

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.705.058>

Effect of Different Levels and Sources of Zinc on Growth Performance and Immunity of Broiler Chicken during Summer

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ABSTRACT

A six week biological experiment was conducted to evaluate different levels and sources of zinc in terms of broiler performance and their immunity under heat stress. A total of 384 broiler chicks were divided into 48 groups with 8 birds in each. Following a 3x4 factorial design 12 dietary treatments were formulated employing three levels of zinc (40, 60, and 80 ppm) from four different sources *viz.* inorganic (IZ), organic (OZ), green nano (GNZ), and market nano zinc (MNZ) and each treatment was allocated four groups. The significantly better body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) was observed at 80 ppm Zn followed by 60 ppm Zn as compared to 40 ppm Zn. The BWG and FCR was ($P < 0.05$) better in birds fed GNZ and poor in birds fed IO, whereas, birds fed MNZ and OZ showed intermediate performance. The overall feed intake of birds was lower ($P < 0.05$) in GNZ and MNZ than IZ or OZ. The results of immune response revealed significantly better ($P < 0.05$) immunity (PHAP and SRBC values) and higher ($P < 0.05$) relative weight of immune organs (thymus, bursa, spleen) of birds fed 80 ppm Zn followed by 60 ppm Zn and then 40 ppm Zn. Among the Zn sources GNZ resulted in higher immunity and organ weights in birds followed by MNZ and lower values were observed in birds fed IZ. The HL ratio in birds fed 80 mg Zn/kg diet was significantly ($P < 0.01$) lower as compared to other two levels which were statistically similar to each other. Among the organic sources GNZ resulted in significantly ($P < 0.01$) higher HL ratio followed by MNZ as compared to IZ and OZ which did not differ significantly from each other. Based on the results it was concluded that 80 ppm GNZ results in better performance and immunity of broiler chicken.

Keywords

Broiler performance, Immunity, Nano zinc, Zinc levels, Zinc sources

Article Info

Accepted:
07 April 2018
Available Online:
10 May 2018

Introduction

Heat stress is a common problem of tropical world associated with poor broiler performance and nutrient utilization (Sahin and Kucuk, 2003a). The higher ambient temperature leads to excess water loss from the birds and the consequent increased mineral excretion is one of the important consequences

of heat distress. Belay and Teeter (1996) have reported lower mineral retention rates in broilers raised at high cycling ambient temperatures (24 to 35°) compared with birds housed at 24°C. The high temperatures impair the availability of minerals, and thus compromise the body weight gain in broiler chicken (Smith *et al.*, 1995). Trace minerals are essential feed additives in the diets of

broiler birds to ensure better health and productivity. The adequate Zn consumption is crucial to the development, maintenance and efficient functioning of the immunological system and the cells associated to it. Zinc has a direct bearing on the growth performance of animals because it is an essential component of both DNA and RNA polymerase enzymes and is vital to the activity of a variety of hormones including glucagon, insulin, growth hormone and the sex hormones.

However there is considerable variability in Zn availability between different Zn sources. The organic form has been found to be more bioavailable than its inorganic form, and hence provides better immunity (Lim and Paik, 2003).

Recently, the trace mineral nutrition of poultry in the form of nanoparticles has elicited considerable interest in poultry nutritionists. Due to the extreme small size and unique physical properties, the nanoparticles are likely to be different when compared to their conventional forms.

As feed additive, these are expected to have the advantage of better bioavailability, small dose rate and stable interaction with other components. The nanoparticles can effectively fulfill the requirement of minerals in the animals, promote growth rate and feed efficiency (Oberdorster *et al.*, 2005).

As a result of large particle surface area low doses of nanoparticles can replace antibiotics as growth promoters, eliminate the antibiotic residues in animal products, and hence reduce the environmental contamination and produce pollution-free animal products (Hett, 2004 and Schmidt, 2009). Thus the current study has employed different Zn levels from different sources for their comparative analysis based on the growth performance and immunity of broiler chicken.

Materials and Methods

Ethical approval

The biological experiment was carried out as per the Institute Animal Ethics Committee's (IAEC) approved schedule (Permission no.: 452/01/ab/CPCSEA).

Experimental design and dietary treatments

Newly hatched CARIBRO-Vishal (384) broiler chicks were divided in 12 treatments. Each treatment consisted of 4 replicates with 8 birds in each. The birds were reared for 42 days under standard management practices.

The birds were reared under high environmental temperature throughout the study period. The experimental groups were T1 fed with 40 ppm inorganic zinc (IZ), T2 fed with 40 ppm organic zinc (OZ), T3 fed with 40 ppm green nano zinc (GNZ), T4 fed with 40 ppm market nano zinc (MNZ), T5 fed with 60 ppm IZ, T6 fed with 60 ppm OZ, T7 fed with 60 ppm GNZ, T8 fed with 60 ppm MNZ, T9 fed with 80 ppm IZ, T10 fed with 80 ppm OZ, T11 fed with 80 ppm GNZ, T12 fed with 80 ppm MNZ. The birds were fed with pre-starter, starter and finisher feeds as per NRC (1994) recommendations to meet their nutritional requirements. A trace mineral mixture was prepared without zinc. Different forms of zinc were added in appropriate quantity to meet the levels of zinc. The experimental feeds were assayed in duplicate (AOAC 1995). Biweekly body weight, feed intake was recorded and feed conversion efficiency was calculated.

Weight of immune organs

Weight of lymphoid organs (thymus, spleen and bursa of Fabricius) was taken at 42nd day of age and expressed as per cent of live weight

Cell mediated immunity

The cell mediated immune response to phytohaemagglutinin type P (PHA-P) was studied by the method of Corrier and Deloach (1990). At the 22nd day post-hatch 0.1 ml (concentration 1 mg/ml) of PHA-P was injected at 3rd and 4th inter-digital space of the right foot.

The left foot will serve as control and was injected with 0.1 ml phosphate buffer (PBS). The foot web index was calculated as the difference between the swelling in the right minus left foot before and after 24 hour of injection and was expressed as millimeter.

Humoral immunity

The antibody response to the SRBC was studied at 29th day post-hatch, wherein 1ml of 1% SRBC will be injected i/v to the birds.

After 5 day post SRBC immunization 2 ml blood was collected from wing vein and the antibody titer was recorded by haemagglutination (HA) titer (Van der Zijpp, 1983 and Siegel and Gross, 1980).

Hematological analysis

Blood samples were collected at 28th and 42th day of feeding experiment. H: L ratio was calculated after blood smear preparation.

Statistical analysis

Data emanated from different treatments were analyzed for statistical significance using completely randomized design (CRD) by following standard methods (Snedecor and Cochran 1989).

Variables having unequal observations were analyzed following least square design method and the Duncan test.

Results and Discussion

Growth performance

The results of growth performance of broiler chicken as affected by feeding of different sources and levels of zinc have been given in Table 1, 2 and 3. The body weight gain of birds fed diet with 80 ppm Zn was significantly ($P<0.01$) higher which was followed by 60 ppm Zn in all the growth phases. Similarly, the body weight gain of birds fed inorganic zinc (IZ) was significantly ($P<0.01$) lower followed by organic zinc (OZ) source, whereas, the Green nano zinc (GNZ) and commercial nano zinc (MNZ) resulted in higher body weight gain of birds. However, the body weight gain of birds fed diet with GNZ and MNZ did not differ significantly. There was a significant ($P<0.01$) effect of zinc levels and zinc source on the body weight gain of birds. Generally, it can be observed that higher body weight gain during 0-14 days and 21-42 days was observed in birds fed 80 ppm Zn diet of either GNZ or MNZ source, whereas, lower weight gain was observed at 40 ppm Zn diet of inorganic source. The feed intake of birds during 0-14 days of age was significantly ($P<0.01$) lower in 40 ppm Zn as compared to 60 and 80 ppm Zn which were statistically similar to each other. However, during 0-42 days significantly ($P<0.01$) lower feed intake was seen at 80 ppm Zn diet as compared to other two levels which were statistically similar to each other. Similarly, in response to zinc sources, significantly ($P<0.01$) lower feed intake was observed in birds fed inorganic zinc and higher intake was seen in birds fed either GNZ or MNZ during 14-21 and 21-42 days of age. During 0-14 days significantly lower feed intake was observed in IZ or GNZ fed birds which was statistically similar to MNZ fed birds as compared to OZ fed birds. During 0-42 days significantly ($P<0.01$) lower feed intake of birds fed OZ was recorded followed by

statistically similar GNZ and MNZ fed birds as compared to IZ fed birds. The interaction between zinc levels and zinc sources had significant effect on the feed intake of birds. Generally, during 14-21 days the birds fed either 60 or 80 ppm Zn diet of either GNZ or MNZ source had higher feed intake as compared to 40 ppm Zn diet of inorganic source, whereas, other combinations of zinc levels and sources resulted in intermediate feed intake values. However, during 0-42 days the birds fed 80 ppm Zn diet of GNZ source had significantly ($P<0.01$) lower feed intake as compared to 40 and 80 ppm Zn diet of inorganic source. The birds fed other combinations of zinc levels and sources had intermediate feed intake values. The FCR of birds have shown significant effects of zinc levels, zinc sources and their interaction. Significantly ($P<0.01$) better feed efficiency was observed in birds fed 80 ppm Zn diet followed by 60 ppm Zn diet and lower feed efficiency was recorded in birds fed 40 ppm Zn diet. Among the zinc sources, generally GNZ resulted in better feed efficiency followed by MNZ, whereas, inorganic zinc source resulted in relatively poor FCR of birds.

The organic zinc was intermediate in terms of feed efficiency of birds. The feed efficiency of birds during 0-14 days and 14-21 days of age was significantly ($P<0.01$) better in birds fed 80 ppm Zn diet of GNZ source which was statistically similar to MNZ source. The significantly poor FCR was observed in birds fed 40 ppm Zn diet of inorganic source followed by 60 ppm Zn diet of inorganic source, whereas, other combinations of levels and sources resulted in intermediate feed efficiency of birds.

Zinc is the main trace mineral involved in metabolism of carbohydrates, lipids, and proteins. The Zn deficiency in animals causes decline of FI, growth, circulating levels of

growth hormone (GH), IGF-I, and decreased hepatic production of IGF-I, GH receptor, and GH binding protein (Sahin and Kucuk 2003b). The administration of nano-minerals is a novel way of providing chicken with quality mineral nutrition. The nano Zn has the capability of bypassing the conventional physiological ways of nutrient distribution and transport across tissues and cell membranes, as well as reaches the targets prior to the destruction. In line with the results of the present study the supplementation of 80 ppm Zn nano zinc diet resulted in improved growth performance (Mohammadi *et al.*, 2015). Similar improvements in body weight gain at various levels of nano Zn have been reported (Ahmadi *et al.*, 2013; Fathi *et al.*, 2016). Mohanan and Nys (1999) has also reported progressive increase in feed intake of broiler chicken with increased dietary levels of Zn until 45 ppm Zn diet was reached. There are the reports of improved feed efficiency of birds when fed nano Zn ranging from 60-90 ppm Zn diet (Ahmadi *et al.*, 2013; Zhao *et al.*, 2014). However, in contrast to this study there are certain reports of non-significant effects of different Zn levels and sources on body weight gain (Yang *et al.*, 2016; Ao *et al.*, 2009), feed intake (Khanagwal *et al.*, 1996; Kidd *et al.*, 1992; Cao *et al.*, 2000), and FCR (Yogesh *et al.*, 2013; Shyam Sunder *et al.*, 2008; Kidd *et al.*, 1992, 1994) of broiler chicken.

Immune response

The results of immune response to feeding of different levels and sources of zinc in broiler chicken are given in Table 4. The PHAP values of males were significantly ($P<0.01$) lower at 40 ppm Zn diet as compared to other two levels which were statistically similar to each other. However, the PHAP values of females and mean PHAP value of birds was higher at 80 ppm Zn diet followed by 60 and lower in 40 ppm Zn diet.

Table.1 Effect of different levels and sources of zinc onBody weight gain

Treatment	0-14	14–21	21-42	0-42
T1	210.37a	168.9a	1052.3	1473.1a
T2	234.45bc	188.0bc	1064.9	1494.0c
T3	310.14f	241.3f	1143.7	1571.2e
T4	308.64f	241.6f	1144.4	1572.0e
T5	227.81b	183.3b	1069.1	1484.0b
T6	244.78d	195.2d	1086.8	1508.2d
T7	334.01g	260.0g	1164.9	1583.5f
T8	334.91g	261.2g	1165.0	1585.4f
T9	238.30cd	190.7cd	1077.0	1487.9b
T10	263.12e	209.1e	1094.8	1511.5d
T11	339.17g	263.2g	1174.5	1595.3g
T12	339.88g	264.2g	1175.2	1595.3g
SEM	7.01	5.09	6.67	6.83
Zinc Levels				
40	265.90a	209.9a	1101.3a	1527.6a
60	285.38b	224.9b	1121.5b	1540.3b
80	295.12c	231.8c	1130.4c	1547.5c
Zinc Source				
IZ	225.49a	181.0a	1066.1a	1481.6a
OZ	247.45b	197.4b	1082.2b	1504.6b
GNZ	327.78c	254.8c	1161.0c	1583.3c
MNZ	327.81c	255.7c	1161.6c	1584.2c
Interaction	0.01	0.01	NS	0.01
Zinc Levels	0.01	0.01	0.01	0.01
Zinc Source	0.01	0.01	0.01	0.01

Table.2 Effect of different levels and sources of zinc on feed intake

Treatment	0-14	14–21	21-42	0-42
T1	317.12a	290.1a	2120.3	2932.5d
T2	335.24b	308.2b	2111.2	2811.8b
T3	349.70cd	322.7c	2155.8	2819.2b
T4	358.00de	331.0d	2157.2	2820.6b
T5	335.46b	308.5b	2116.9	2925.7cd
T6	335.93b	308.9b	2119.3	2804.1b
T7	368.25ef	341.3e	2160.9	2821.7b
T8	371.74f	344.7e	2161.1	2825.2b
T9	335.98b	309.0b	2129.8	2893.5c
T10	345.34bc	318.3c	2129.3	2805.7a
T11	367.12ef	340.1e	2161.0	2744.2b
T12	370.45f	343.5e	2162.4	2805.7b
SEM	2.56	2.56	3.19	7.93
Zinc Levels				
40	340.01a	313.0a	2136.1	2846.0b
60	352.85b	325.8b	2139.6	2844.2b
80	354.72b	327.7b	2145.6	2812.3a
Zinc Source				
IZ	329.52a	302.5a	2122.3a	2917.2c
OZ	338.84b	311.8b	2120.0a	2786.7a
GNZ	361.69c	334.7c	2159.2b	2815.5b
MNZ	366.73c	339.7c	2160.3b	2817.1b
Interaction	0.01	0.05	NS	0.05
Zinc Levels	0.01	0.01	0.05	0.01
Zinc Source	0.01	0.01	0.01	0.01

Table.3 Effect of different levels and sources of zinc onFCR

Treatment	0-14	14–21	21-42	0-42
T1	1.51h	1.72h	2.02	1.99
T2	1.43f	1.64f	1.98	1.88
T3	1.13bc	1.34bc	1.89	1.79
T4	1.16c	1.37c	1.89	1.79
T5	1.47g	1.68g	1.98	1.97
T6	1.37e	1.58e	1.95	1.86
T7	1.10ab	1.31ab	1.86	1.78
T8	1.11ab	1.32ab	1.86	1.78
T9	1.41f	1.62f	1.98	1.94
T10	1.31d	1.52d	1.95	1.82
T11	1.08a	1.29a	1.84	1.76
T12	1.09a	1.30a	1.84	1.76
SEM	0.02	0.023	0.009	0.012
Zinc Levels				
40	1.31c	1.52c	1.94c	1.87c
60	1.26b	1.47b	1.91b	1.85b
80	1.22a	1.43a	1.90a	1.82a
Zinc Source				
IZ	1.46d	1.67d	1.99c	1.97c
OZ	1.37c	1.58c	1.96b	1.85b
GNZ	1.10a	1.31a	1.86a	1.78a
MNZ	1.12b	1.33b	1.86a	1.78a
Interaction	0.01	0.01	NS	NS
Zinc Levels	0.01	0.01	0.01	0.01
Zinc Source	0.01	0.01	0.01	0.01

Table.4 Effect of different levels and sources of zinc on Immunity and HL ratio

Treatment	PHAP	SRBC	HL ratio 28 day	HL ratio 42 day
T1	0.22	6.38a	0.44	0.47
T2	0.28	7.16d	0.38	0.41
T3	0.38	7.36f	0.35	0.38
T4	0.35	7.36f	0.35	0.38
T5	0.23	6.45b	0.42	0.46
T6	0.31	7.19de	0.36	0.40
T7	0.39	7.43g	0.33	0.37
T8	0.38	7.43g	0.33	0.35
T9	0.25	6.49c	0.39	0.41
T10	0.31	7.22e	0.33	0.35
T11	0.40	7.47h	0.30	0.32
T12	0.39	7.47h	0.30	0.32
SEM	0.01	0.06	0.01	0.01
Zinc Levels				
40	0.31a	7.06a	0.38	0.41
60	0.33b	7.12b	0.36	0.39
80	0.34c	7.16c	0.33	0.35
Zinc Source				
IZ	0.23a	6.44a	0.41	0.44
OZ	0.30b	7.19b	0.36	0.39
GNZ	0.39d	7.42c	0.33	0.36
MNZ	0.37c	7.42c	0.32	0.35
Interaction				
Zinc Levels	0.01	0.01	0.01	0.01
Zinc Source	0.01	0.01	0.01	0.01

Table.5 Effect of different levels and sources of zinc on Immune organ weight

Treatment	Thymus	Bursa	Spleen
T1	0.300	0.135	0.110
T2	0.340	0.173	0.143
T3	0.390	0.238	0.210
T4	0.388	0.239	0.225
T5	0.320	0.151	0.115
T6	0.356	0.188	0.158
T7	0.408	0.260	0.230
T8	0.410	0.251	0.220
T9	0.343	0.164	0.133
T10	0.373	2.805	0.183
T11	0.423	0.275	0.245
T12	0.424	0.279	0.235
SEM	0.006	0.217	0.007
Zinc Levels			
40	0.354a	0.196	0.171a
60	0.373b	0.213	0.180a
80	0.390c	0.881	0.198b
Zinc Source			
IZ	0.321a	0.150	0.119a
OZ	0.356b	1.055	0.161b
GNZ	0.407c	0.258	0.228c
MNZ	0.407c	0.256	0.227c
Interaction	NS	NS	NS
Zinc Levels	0.01	NS	0.01
Zinc Source	0.01	NS	0.01

The PHAP value of birds fed GNZ was significantly ($P<0.01$) higher followed by MNZ and lower in birds fed IZ followed by OZ. However, in males the PHAP values of birds fed GNZ and MNZ did not differ significantly from each other. The interaction effect of levels and sources of zinc has shown that in general significantly higher PHAP values were recorded in birds fed either 40, 60 or 80 ppm Zn diet of GNZ source followed by feeding of either 40, 60 or 80 ppm Zn diet of MNZ source as compared to 40 ppm Zn diet of inorganic source. The other combinations of levels and sources resulted in intermediate values of PHAP. Similarly, the antibody response to SRBC was significantly ($P<0.01$) higher in birds fed 80 ppm Zn diet followed by 60 ppm Zn diet and lower in 40 ppm Zn diet. The SRBC value was significantly ($P<0.01$) lower in birds fed inorganic zinc followed by birds fed OZ and higher values were observed in birds fed either GNZ or MNZ. The interaction effect has shown that 80 ppm Zn diet of either GNZ or MNZ source has resulted in higher SRBC response in males and means SRBC response followed by 60 ppm Zn diet of either GNZ or MNZ source, whereas, other combinations of levels and sources yielded intermediated SRBC responses.

Zinc plays an important role in immunomodulation by increasing the counts of thymocytes and peripheral T cells and by enhancing the production of interferon (Kidd *et al.*, 1996). Chunshanzhang *et al.*, (2006) and Shyam Sunder *et al.*, (2008) recommend 80 ppm Zn diet for better immune response of the broiler chicken. Further, Bartlett *et al.*, (2003) observed higher antibody titers to SRBC at 181 ppm Zn of Zn compared with 34 to 68 ppm Zn supplementation and Bertuzzi *et al.*, (1997) reported higher response of T-dependent humoral immunity in chickens fed 160 ppm Zn. In this study it may be due to the different genetic stock and use of nano Zn

that better immune response was observed at lower levels as well. The higher production of interleukin-2 at higher Zn levels was proposed to be the reason for better cell mediated immunity of broiler chicken (Kidd *et al.*, 1996).

Response to heat stress

Effect of different levels and sources of zinc on H: L ratio was given in Table 4. The HL ratio in birds fed 80 ppm Zn diet was significantly ($P<0.01$) lower as compared to other two levels which were statistically similar to each other. Among the organic sources GNZ resulted in significantly ($P<0.01$) higher HL ratio followed by MNZ as compared to IZ and OZ which did not differ significantly from each other. The interaction effect between the zinc source and zinc levels yielded significantly ($P<0.01$) higher HL ratio in birds fed 40 or 80 ppm Zn diet of GNZ source followed by 60 ppm Zn of BNZ source, however, lower values were observed in birds fed 40 ppm Zn of either organic or inorganic source, and 60 or 80 ppm Zn diet of inorganic source. The dietary treatments yielded intermediate values.

Zinc plays vital role in the phosphorylation of proteins mediated by protein kinase C (Zalewski *et al.*, 1993) and the subsequent changes through protein phosphorylation regulate activation and proliferation heterophils, monocytes and thrombocytes which are capable of phagocytosis in avian species. Moreover, Zn is an essential co-factor for thymulin that is a thymic hormone binds to surface receptors of lymphocyte T and leads to maturation and activation of these cells. In acute inflammatory responses to infectious as well as non-infectious causes, heterophils are one of the first phagocytes to accumulate in the affected tissue (Maxwell and Robertson, 1998). The HL ratio is a sensitive and convenient index for

measurement of stress in general (Mashaly *et al.*, 2004) and adrenal-corticoid hyperactivity in particular in birds. There has been the report of linear increase of lymphocyte (%) and decrease of HL ratio with increasing Zn levels (Rouhalaminiet *al.*, 2014). However, Mohammadi *et al.*, (2015) have reported an increase of heterophil (%) with no significant effect on the HL ratio of birds due to Zn supplementation. Further Sajadifar (2013) has reported decrease of HL ratio with increase of Zn levels in diet, indicating lower impact of stressors on broiler chicken, and Shyam Sunder *et al.*, (2008) suggested that supplementation of Zn was useful in reducing HL ratio in young broilers. To our knowledge there is no report of HL ratio in response to nano Zn feeding in poultry.

Immune organ weights

The results of immune organ weights as affected by different zinc levels and source has been given in Table 5. The weight of thymus and bursa (in males only) was recorded significantly ($P<0.01$) higher in birds fed 80 ppm Zn diet followed by 60 ppm Zn diet and lower values were observed at 40 ppm Zn diet. The spleen weight was significantly ($P<0.01$) higher in birds fed 80 ppm Zn as compared to other two levels which were statistically similar to each other. Similarly, the weight of thymus, bursa (in males only), and spleen was significantly ($P<0.01$) lower in birds fed inorganic zinc followed by birds fed organic zinc as compared to the birds fed GNZ or MNZ, which were statistically similar to each other. There was no interaction effect of zinc levels and sources on the weight of thymus, bursa, and spleen.

It is obvious for the fact that when general body weight is improved relatively the weight of body organ is improved. The results of this study pertaining to the relative weight of

immune organs are corroborated by earlier reports where diets deficient in zinc led to the atrophy of thymus (Prasad and Oberleas, 1971) and reduction in spleen weight of rats (Mengheri *et al.*, 1988). The relative reduction in size of lymphoid organs have been reported by Kidd *et al.*, (1996) with the possible decrease in T-cell function. However, in contrast to our findings Akbari *et al.*, (2008) reported that 60 ppm Zn basal diet had no significant effect on relative weight of liver and bursa but significantly increased relative weight of spleen as compared to other dietary groups. Further, Bartlett and Smith (2003) reported that Zn supplementation in broiler chicks did not affect the relative weights of bursa, spleen and thymus but improved the primary and secondary antibody titers.

Thus it was concluded that 80 ppm of zinc was optimum during summer and nano zinc (NZ) proved to be better source than inorganic or organic sources of zinc for improved performance and immunity of broiler chickens during summer. Considering production ease and cost green nano zinc would be recommended.

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How to cite this article:

Dukare Sagar, P., A.B. Mandal, Nasir Akbar and Dinani, O.P. 2018. Effect of Different Levels and Sources of Zinc on Growth Performance and Immunity of Broiler Chicken during Summer. *Int.J.Curr.Microbiol.App.Sci*. 7(05): 459-471. doi: <https://doi.org/10.20546/ijcmas.2018.705.058>